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Award Number: W81XWH-08-1-0252

TITLE: Epigenetic Regulation of Ovarian Tumor Immunity

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REPORT DATE: November 2009

TYPE OF REPORT: Revised Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE November 2009		2. REPORT TYPE Revised Annual		3. DATES COVERED 1 May 2008 – 31 October 2009	
4. TITLE AND SUBTITLE Epigenetic Regulation of Ovarian Tumor Immunity				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-08-1-0252	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Protul A. Shrikant, Ph.D. E-Mail: Protul.Shrikant@RoswellPark.org				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Health Research, Inc. Buffalo, NY 14263				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The standard of care has produced marginal extension of remission rates in ovarian cancer. Based on preliminary observations that epigenetic regulators like Trichostatin A (TSA) can reduce ovarian tumor burden in a syngeneic murine model system, we tested the notion that TSA treatment leads to dendritic cell maturation that promotes effector CD8+ T cell over CD4+ T regulatory cell generation. At 10 mg/kg/day for 5 times on alternate days, TSA was able to control ovarian tumor growth, however the tumor growth resumed by day 40. Surprisingly, 1-MT; a known inhibitor of indole-amine 2,3-dioxygenase (IDO) was unable to cause ovarian tumor growth inhibition, which was attributed to its inability to block IDO expression in the ovarian tumor microenvironment. However, TSA mediated ovarian tumor control required MHC Class 1 dependent CD8+ T cells. These studies have identified the role of CD8+ T cells in promoting ovarian tumor immunity and delineated the ability of TSA mediated epigenetic alteration to enhance CD8+ T cell mediated ovarian tumor control. Ongoing studies are focused on testing new generation of IDO inhibitors for ovarian cancer.					
15. SUBJECT TERMS Trichostatin A (TSA), Epigenetic modifiers, Indole-amine 2,3 Dioxygenase (IDO), CD4+, CD8+, FoxP3+, Regulatory T cells, Effector T cells, immunity, ovarian tumor, MOSEC.					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	6	19b. TELEPHONE NUMBER (include area code)

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1. INTRODUCTION: Ovarian cancer continues to be the leading cause of death among gynecological cancers. The 5-year survival rate for women diagnosed with late stage of ovarian cancer is 31% and durable remission is a highly desirable unmet goal (1). The use of traditional modalities to treat ovarian cancer has not changed the rate of recurrence and/or the remission rates. Emerging evidence indicates that adaptive immunity mediated by CD8⁺ T cells can play an important role in controlling ovarian tumor growth, whereby inducing interest in developing approaches to augment effective CD8⁺ T cell responses in ovarian cancer patients and extend remission rates (2). However, the fact that the relative frequency of CD4⁺ T regulatory cells and effector CD8⁺ T cells in ovarian cancer patients as well as in murine models is predictive of beneficial outcomes, behooves developing targeted strategies to cause skewing of T cell responses to favor tumor regressions. In preliminary studies, we have noted the ability of the epigenetic modifier trichostatin A (TSA) to extend survival of ovarian tumor bearing mice. Based on this observation, we hypothesize that TSA caused alteration of dendritic cell maturation whereby leading to decreased CD4⁺ T regulatory cell generation and enhanced effector CD8⁺ T cells, leading to enhanced ovarian tumor control and perhaps durable immunity. The information obtained will validate a novel approach for ovarian cancer therapy and by delineating mechanisms we are likely to arrive at a meaningful strategy for clinical development.

2. BODY:

Aim 1: To test whether TSA treatment reverses the generation of CD4⁺ T regulatory cells in MOSEC tumor bearing hosts. Specific Tasks: In the syngeneic murine ovarian tumor (MOSEC) model, to determine the impact of TSA treatment on DC maturation and T reg. generation,

1. Groups of 10 age matched C57BL/6 (B6) mice bearing murine ovarian serous epithelial carcinoma (MOSEC) tumor were administered TSA (10 mg/kg/day X 5 times on alternate days) by intraperitoneal injection on day 10 post-tumor challenge.
2. The cells harvested from the tumor draining LN's, spleen and the tumor site starting on day 2 after the final TSA administration will be subjected to phenotypic analysis by staining followed by flow cytometry and functional evaluation for regulatory capacity in a standard in vitro MLR reaction. To determine whether tumor induced immature DC's to promote T reg. generation, we will perform To determine whether tumor induced immature DC's generate T reg. cells in vivo, we will use 1-MT (1-methyl tryptophan)(20 microg/day x 50 days in drinking water) treatment in vivo starting on day 10 of tumor challenge.

Results: In the past year, we have established that:

1. The administration of HDACi (Trichostatin A) to ovarian tumor bearing mice can significantly reduce the frequency of tumor induced FoxP3⁺/ CD4⁺/CD25⁺ regulatory T cells in our syngeneic murine tumor (MOSEC) model (Figure 1, TSA versus PBS). However, the TSA mediated inhibition of Treg. generation was transient and by day 60 re-emergence of Treg. cells was noted.

Discussion of Results: The ability of TSA to block induction of T regulatory CD4+ T cells in MOSEC ovarian tumor bearing animals is encouraging and confirms the précis for our study, which is that epigenetic modification can be used to alter host T cell immune responses. However, the lack of persistence in efficacy indicates that the epigenetic modifier may only transiently reduce the DC maturation status and that continued administration and/or careful dose response of TSA needs to be performed and evaluated to achieve durable responses. This may not be feasible as TSA does not specifically affect DC and T cells and its off target effects may result in overt toxicity.

2. Surprisingly, the inhibition of IDO by 1-MT administration in this ovarian tumor model system failed to inhibit Treg. generation (Figure 1; PBS versus 1-MT).

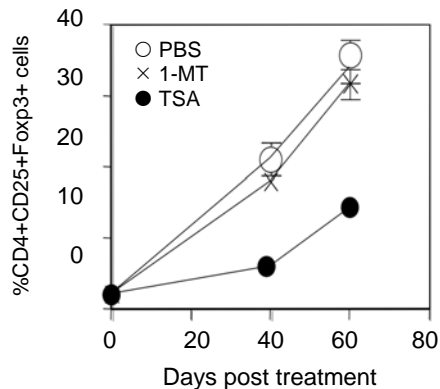


Figure 1: HDACi but not IDO blockade reduces ovarian tumor induced T regulatory cells.

Groups of three mice bearing ovarian tumor (MOSEC) were treated with either PBS (control), or Trichostatin A (TSA, 10 mg/kg/day X 5) or 1-Methyl tryptophan (1-MT, 10 microg/day x 50 days). The draining lymph nodes from the mice were harvested and evaluated by flow cytometry for the frequency of CD4+ T regulatory cells (CD4+/CD25+/FoxP3+) on indicated days. The error bars represent SD of mean values obtained from 3 mice/group and a representative graph from 3 experiments is shown.

Discussion of results: The failure of 1-MT mediated IDO blockade to inhibit the ovarian tumor induced CD4+/CD25+/FoxP3+ regulatory T cells could be due to several factors including the possibility that ovarian tumor induced T regulatory cells utilize other molecular pathways such as arginase and/or STAT3, which should be tested. It could also be envisioned that the 1-MT was ineffective at blocking IDO expression and/or activity, which has been recently reported (3) and new second-generation IDO blocking reagent are undergoing pre-clinical evaluation and should be tested in our ovarian tumor model system

3. KEY RESEARCH ACCOMPLISHMENTS: Our studies are the first to demonstrate the ability of TSA to inhibit CD4+ T regulatory cells. However, the notion that this is mediated by reducing indoleamine 2,3-dioxygenase (IDO) may not be accurate and other pathways for T regulatory cell generation may be operational in ovarian tumor models.

4. REPORTABLE OUTCOMES: The ability of TSA to enhance survival of ovarian tumor bearing host demonstrates the potential use for clinical benefit. However, comprehensive studies directed at determining the dose and duration of TSA administration for optimal/durable benefits in the pre-clinical murine models are required to generate protocols for Phase 1 clinical trials.

5. CONCLUSIONS: The epigenetic modifier TSA enhances survival of ovarian tumor bearing host demonstrating its potential use for clinical benefit.

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